

WHAT IS CLAIMED IS:

1. A process for preparing a vascular endothelial growth factor (VEGF) dimer comprising:

providing transformed host bacterial cells, wherein the transformed host bacterial cells comprise an exogenous nucleic acid encoding an amino acid sequence of a VEGF monomer operably linked to a promoter, wherein the amino acid sequence has at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1 and wherein the amino acid sequence is extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the VEGF dimer by the bacterial host cell, and the amino acid sequence retains a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116);

culturing said host cells under conditions suitable for expression of said VEGF monomer, whereby a first VEGF monomer and a second VEGF monomer are produced;

forming the VEGF dimer from the first and second VEGF monomers; and
recovering said VEGF dimer.

2. The process of claim 1, wherein n is 1.

3. The process of claim 2, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

4. The process of claim 3, wherein AA represents a lysine (Lys) residue.

5. The process of claim 1, further comprising the step of purifying said VEGF dimers.

6. The process of claim 5, further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

7. The process of claim 6, wherein removal is performed by enzymatic digestion.

8. The process of claim 7, wherein diaminopeptidase is used to perform the enzymatic digestion.

9. The process of claim 1, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.

10. The process of claim 1, further comprising the step of refolding said VEGF dimers.

11. The process of claim 10, wherein refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in a ratio to each other sufficient to produce the desired mixture of VEGF dimers.

12. A process for producing a vascular endothelial growth factor (VEGF) dimer composed of two VEGF monomers, in which each monomer comprises amino acids 11 to 116 of SEQ ID NO: 1, or comprises an amino acid sequence having at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, and retaining a cysteine (Cys) at a position corresponding to position 116 of SEQ ID NO: 1 (Cys-116), where Cys-116 of each monomer is disulfide bonded to an additional extraneous Cys, comprising the steps of:

providing transformed bacterial host cells comprising a species of exogenous nucleic acid encoding a promoter operably linked to a polypeptide of SEQ ID NO: 1 extended by a Met(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, wherein at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of blocking the proteolytic degradation of the mature N-terminus of the VEGF polypeptides by the bacterial host cell;

culturing said bacterial host cells under conditions suitable for expression of said exogenous nucleic acid and the synthesis of said N-terminally-extended VEGF monomers, and recovering said VEGF dimer.

13. The process of claim 12, wherein n is 1.

14. The process of claim 13, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

15. The process of claim 14, wherein AA represents a lysine (Lys) residue.

16. The process of claim 12, further comprising the step of purifying said VEGF dimer.

17. The process of claim 16, further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

18. The process of claim 17, wherein removal is performed by enzymatic digestion.

19. The process of claim 18, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.

20. The process of claim 12, additionally comprising the step of refolding said VEGF dimer.

21. The process of claim 14, additionally comprising the step of refolding said VEGF dimer.

22. The process of claim 17, additionally comprising the step of refolding said VEGF dimer.

23. The process of claim 22, wherein refolding is performed in a refolding buffer comprising cysteine and cystine.

24. A process for preparing a vascular endothelial growth factor (VEGF) dimer comprising:

providing host cells, wherein the host cells comprise an exogenous nucleic acid encoding an amino acid sequence of a VEGF monomer operably linked to a promoter, wherein the amino acid sequence has at least about 90% sequence identity with amino acids 11 to 116 of SEQ ID NO: 1, retains a cysteine (Cys) at or corresponding to position 116 of SEQ ID NO: 1 (Cys-116), and wherein at least one monomer has an Asn-to-Glu amino acid substitution at or corresponding to position 75 of SEQ ID NO: 1;

culturing said host cells under conditions suitable for expression of said VEGF monomer, whereby a first VEGF monomer and a second VEGF monomer are produced;

forming the VEGF dimer from the first and second VEGF monomers; and

recovering said VEGF dimer.

25. The process of claim 24, wherein each monomer comprises amino acids 1 to 120 of SEQ ID NO: 1.

26. The process of claim 24, wherein monomer comprises amino acids 1 to 121 of SEQ ID NO: 1.

27. The process of claim 24, wherein at least about 95% of said VEGF dimers are devoid of an N-terminal methionine residue.

28. The process of claim 24, wherein the Cys residue corresponding to Cys-116 of SEQ ID NO:1 of each monomer is disulfide bonded to an extraneous Cys.

29. The process of claim 24, wherein the Cys residue corresponding to Cys-116 of SEQ ID NO:1 of the two monomers are interconnected with an interchain disulfide bond.

30. The process of claim 24, wherein the Cys residue corresponding to Cys-116 of SEQ ID NO:1 of one or both monomers is not reduced.

31. The process of claim 24, additionally comprising the step of purifying said dimers.

32. The process of claim 24, wherein said transformed host cells are bacterial cells.

33. The process of claim 32, wherein said bacterial cells are *E. coli* cells.

34. The process of claim 32, wherein the exogenous nucleic acid encodes a polypeptide of SEQ ID NO: 1 extended by a Met-(AA)_n- sequence at the amino terminus (N-terminus), wherein Met stands for methionine, n is 1-7, and AA represents identical or different amino acids, where at least one of the AA amino acids, or a combination of two or more of the AA amino acids, is capable of retarding proteolytic degradation of the mature N-terminus of the VEGF dimer by the bacterial host cell.

35. The process of claim 34, wherein n is 1.

36. The process of claim 35, wherein AA represents an amino acid selected from the group consisting of lysine (Lys) and arginine (Arg) residues.

37. The process of claim 36, wherein AA represents a lysine (Lys) residue.

38. The process of claim 34, further comprising the step of purifying said VEGF dimers.

39. The process of claim 38, further comprising the removal of the N-terminal Met(AA)_n- sequence following at least partial purification.

40. The process of claim 39, wherein removal is performed by enzymatic digestion.

41. The process of claim 32, further comprising the step of refolding said VEGF dimers.

42. The process of claim 41, wherein refolding is performed in a refolding buffer comprising cysteine and cystine in amounts and in a ratio to each other sufficient to produce the desired mixture of VEGF dimers.